*sustainability*

MDPI

*Article*

# Optimization of Xanthan Gum Production by Demerara Sugar Using Response Surface Methodology

**Larissa Castor Ramos [1], Meirielly Santos Jesus [2,*], Preciosa Pires [2], Alberto S. Fontes-Junior [3], Erica S. Nunes [3], Klebson S. Santos [3], José António Teixeira [4], Francine Ferreira Padilha [3], Denise Santos Ruzene [1,5] and Daniel Pereira Silva [1,5]**

1 Northeastern Biotechnology Network (RENORBIO), Federal University of Sergipe, São Cristóvão 49100-000, SE, Brazil; silvadp@hotmail.com (D.P.S.)
2 Center for Research and Development in Agri-Food Systems and Sustainability, Polytechnic Institute of Viana do Castelo, Praça General Barbosa, 4900-347 Viana do Castelo, Portugal
3 Tiradentes University, Campus Farolândia, Aracaju 49032-490, SE, Brazil
4 Centre of Biological Engineering, University of Minho, Campus Gualtar, 4710-057 Braga, Portugal
5 Center for Exact Sciences and Technology, Federal University of Sergipe, São Cristóvão 49100-000, SE, Brazil
* Correspondence: meiriellyjesus@ipvc.pt

**Abstract:** Xanthan gum (XG) production using three *Xanthomonas* sp. strains (290, 472, and S6) was evaluated by applying a $2^3$ full factorial central composite design response to study the interactive effects of the fermentation medium component concentrations as parameters to determine the efficiency of the gum production in batch experiments. The experimental variables were the carbon source (demerara sugar or sucrose), potassium phosphate dibasic, and magnesium sulfate. Experimental results showed the $K_2HPO_4$ concentration as the important parameter for XG production by using *Xanthomonas axonopodis* pv. *manihotis* IBSBF 290 and *X. campestris* pv. *campestris* IBSBF 472, while for the *Xanthomonas* sp. S6 strain, the $MgSO_4 \cdot 7H_2O$ concentration was the determining factor in XG production using demerara sugar or sucrose as a carbon source. The strains of *Xanthomonas* 472 and S6, using demerara sugar and higher concentrations of salts, exhibited a higher yield of XG (36 and 32%) than when using sucrose and the same concentration of salts. The experimental outcomes highlighted demerara sugar as a suitable and efficient alternative carbon and micronutrient source for XG production. Despite the bacterial strain influence, the medium composition is crucial for this fermentation process. Therefore, the evaluated salts are important factors for XG production, and the demerara sugar can partially replace this mineral salt requirement as indicated by the face-centered composite experimental design due to its chemical composition. Overall, demerara sugar provides promising properties for XG production.

**Keywords:** xanthan gum; demerara sugar; mineral salts; response surface methodology; fermentation

check for updates

**Citation:** Ramos, L.C.; Jesus, M.S.; Pires, P.; Fontes-Junior, A.S.; Nunes, E.S.; Santos, K.S.; Teixeira, J.A.; Padilha, F.F.; Ruzene, D.S.; Silva, D.P. Optimization of Xanthan Gum Production by Demerara Sugar Using Response Surface Methodology. *Sustainability* **2023**, *15*, 5080. https://doi.org/10.3390/su15065080

Academic Editor: Idiano D'Adamo

Received: 14 February 2023
Revised: 4 March 2023
Accepted: 9 March 2023
Published: 13 March 2023

**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

## 1. Introduction

Xanthan gum (XG) is a water-soluble microbial exopolysaccharide (EPS) produced by bacteria of the genus *Xanthomonas* [1]. The monomeric structure of the XG is formed by two units of glucose (cellobiose) bonded in the main chain (the backbone) with a branch formed by two units of mannose and one unit of glucuronic acid (Figure 1) [2,3]. The XG is an EPS, and a biopolymer, chemically characterized by a structural chain with a molar mass ranging between $2 \times 10^6$ and $20 \times 10^6$ Da [4,5]. Furthermore, its molecular weight is influenced both by bacterium strains and fermentation conditions, producing a variety of gums of industrial interest [6]. These aspects are important since the XG molecular structure and conformational state are closely related to its rheology, stability, and function [7,8]. These properties allow its application as a thickening, dispersant, emulsifier, and viscous aqueous solution at low concentrations (0.05–1%), and are stable over a wide range of pH values

and temperatures. Moreover, XG is a dry, tasteless, white-to-cream, and biodegradable powder, and is less expensive compared to synthetic polymers [9].

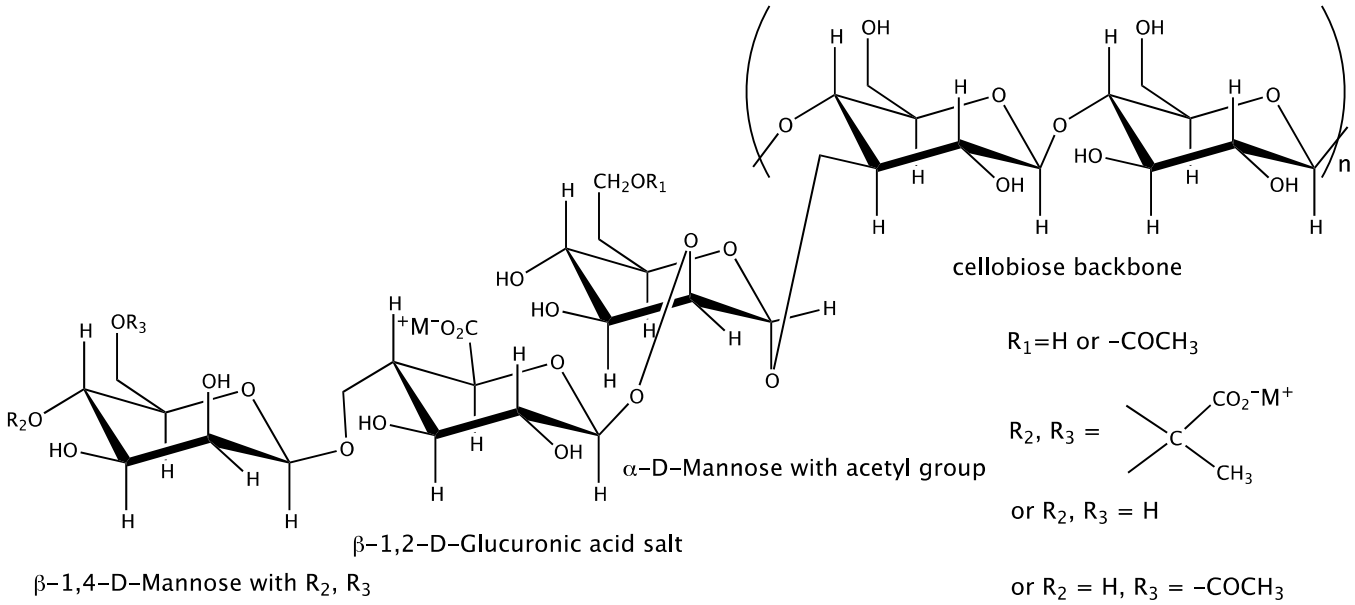


**Figure 1.** The structure of the xanthan gum molecule.

These properties add industrial value and explain the wide commercial acceptance of this polysaccharide, whereby the xanthan market is constantly expanding to meet its global demand [7,10]. The global XG market grows at a significant rate of 5.6% since 2019, predicting that its market value will reach USD 1.2 billion by 2030 [11]. Its demand increase is due to the growth in processed food consumption, and applications in the agrochemical, cosmetics, driller fluid, and foam stabilizer segments [7]. This commercial demand is a key factor that stimulates studies to increase xanthan production on an industrial scale by sustainable processes to exploit the micro-organism potential [12,13].

Therefore, studies have been carried out analyzing the variables involved in XG production to obtain efficient results, since this productivity can be affected by biotic and abiotic factors [4,7]. Fermentation depends on many parameters and variables, including micronutrients (e.g., potassium, iron, and calcium salts), macronutrients such as carbon and nitrogen sources, oxygen transfers, agitation rate, pH, temperature, and fermentation duration beyond the *Xanthomonas* strain production efficiency [14,15]. These conditions represent a pivotal point in XG production by affecting the fermentative medium cost, further promoting changes in the gum characteristics, downstream processes, and, consequently, in its productivity [7,16]. In addition, the adjustment of the strains with the conditions applied in the fermentation are important as they directly influence the characteristics, techniques, yields, compositions, and structures [7,10] of the XG produced. The different strains present different productivity levels depending on the factors implemented during the fermentation [17] The *Xanthomonas* strains used to produce XG that are most reported in the literature are *X. campestris* pv. *campestris* 629, *X. campestris* pv. *campestris* 1078, *X. campestris* pv. *campestris* S6, *X. campestris* pv. *campestris* 254, *X.* sp. 1537, *X. campestris* pv. *campestris* 729, *X. campestris* pv. *campestris* 607, *X. campestris* pv. *campestris* 1167, *X. campestris* pv. *mangiferaeindicae* 1230, *X. campestris* pv. *arracia* 1198, *X. axonopodis* pv. *manihotis* 1182, and *X. melonis* 68 [18,19].

On an industrial scale, glucose and sucrose are the usual carbon sources for XG production. Considering that the raw material cost is a factor which burdens its production, alternative carbon sources have been evaluated to obtain this polysaccharide [20–22]. Regarding Brazil, sucrose as a carbon source is a favorable point for its production since its soil and climate allow an intense development of sugarcane cultivation [23]. Therefore,

the binomial XG–sugarcane exhibits a positive effect on profitable income for the agro-industrial market.

In this regard, sugarcane juice is a raw material option for this gum production due to the high content of sucrose, minerals, and vitamins [24]. This broth is used to produce demerara sugar, which, after a brief refinement process, still maintains its rich chemical composition compared to refined sugar [25]. Considering its cost of production in Brazil [26], demerara sugar is an alternative carbon source for XG production due to its nutritional profile and little processing [27]. In addition, as it is an abundant, low-cost, and little-processed product, it can be considered an economical solution and a possible low-cost approach to the production of XG, which can contribute to a sustainable bioeconomy [28].

Nonetheless, several variables affect xanthan production, including technical, biochemical, and microbiological aspects, whose evaluation is crucial for potential industrial implementation [12]. Hence, experimental designs and related statistical methods have been applied systematically to investigate different problems correlating to development and production [15,29,30]. These designs include blocking and factorial experiments to determine the steepest climb ascent path to identify the individual factor effect and approach the neighborhood of the optimal response [17,31,32].

Screening research on alternative or conventional fermentation media for this polymer synthesis has previously been reported [7,33], for example, agricultural residues and biomass with lignocellulosic content [20] (such as tapioca pulp [34], sugarcane bagasse [35], orange peel [36], kitchen waste [37], rice bran [38], chicken feathers [39], coconut shell, cocoa husk [20], potato crop [40], cellar wastewater [41], and corncob [19]); however, these studies found some limitations in the pre-treatments and production yields. Furthermore, there are no studies reporting and describing the use of demerara sugar in XG production. This raw sugar appears to be a promising source since its chemical composition displays adequate nutritional requirements for a fermentative medium, allowing it to be an excellent substrate alternative for the industrial production of XG.

Therefore, the objective of the research was to design and compare XG production processes and thus maximize its yield through a full central factorial composite design ($2^3$). Furthermore, the interactive effects of $MgSO_4$, $K_2HPO_4$, and the carbon source (camera sugar or sucrose) as variable parameters of the fermentation medium using three strains of *Xanthomonas* will be evaluated, thus finding ideal conditions for the biosynthesis of XG, with a greater yield and using an alternative carbon source to obtain the most effective operating conditions, and being able to contribute to a bioeconomy sustainable.

## 2. Materials and Methods

### *2.1. Strains*

*Xanthomonas axonopodis* pv. *manihotis* IBSBF 290, *X. campestris* pv. *campestris* IBSBF 472, and *Xanthomonas* sp. S6 from the Cultures Collection of the Biomaterials Laboratory (Institute of Technology and Research, Aracaju, SE, Brazil) were used as xanthan-producing micro-organisms. Bacteria were grown on MY-agar medium with the following composition ($g{\cdot}L^{-1}$)—yeast extract, 3.0; malt extract, 3.0; peptone, 5.0; glucose, 10.0; and agar, 20.0—and subcultured every three weeks. After incubation (25 °C/48 h), cultures were stored at 4 °C [42]. Inoculum cultures were prepared in MY liquid medium in 125 mL Erlenmeyer flasks containing 14 mL of culture medium at 28 °C, at 100 rpm for 24 h until reaching a log growth of $10^{11}$ CFU/mL [43].

### *2.2. Xanthan Gum Production*

The xanthan production was performed in 250 mL Erlenmeyer flasks containing 85 mL of the culture medium under experimental conditions of 28 °C, 96 h, and 180 rpm. The fermentation medium was constituted by the carbon source (demerara sugar or sucrose) ranging from 30 to 70 $g{\cdot}L^{-1}$, supplemented with $MgSO_4{\cdot}7H_2O$ (ranging from 0.2 to 1.0 $g{\cdot}L^{-1}$), $K_2HPO_4$ (ranging from 0.01 to 1.0 $g{\cdot}L^{-1}$), $(NH_4)_2SO_4$ (2.2 $g{\cdot}L^{-1}$), $H_3BO_3$ (0.0066 $g{\cdot}L^{-1}$, $FeCl_3$ (0.0026 $g{\cdot}L^{-1}$), $CaCl_2$ (0.0022 $g{\cdot}L^{-1}$), and $ZnSO_4$ (0.0022 $g{\cdot}L^{-1}$). Fer-

mentation started with an inoculum concentration of 15% (*v*/*v*), according to medium composition conditions established by experimental design.

### *2.3. Xanthan Gum Recovery*

After fermentation, the samples were subjected to centrifugation (9626× *g*/5 °C/60 min) to remove cells from the fermented broth. Then, the cell-free supernatant was precipitated with 98% ethanol at a 3:1 (*v*/*v*) ratio for biopolymer recovery. After recovery, the precipitated gum was collected and dried at 30 ± 2 °C for 72 h. Its production was expressed as $g \cdot L^{-1}$ (grams of XG per liter of fermentation broth) [42].

### *2.4. Experimental Design and Data Analysis*

For each *Xanthomonas* strain used experimentally, a $2^3$ full factorial central composite design was used to optimize XG production, with variable concentrations of $MgSO_4 \cdot 7H_2O$ (0.2–1 $g \cdot L^{-1}$), $K_2HPO_4$ (0.01–1 $g \cdot L^{-1}$), and demerara sugar or sucrose (30–70 $g \cdot L^{-1}$). Table 1 shows the conditions of the performed experiments. All experiments were conducted in triplicate. For statistical calculation, factor levels (in coded values) were −1, 0, and +1, where 0 corresponded to the central point. The range and levels of the variables investigated in this study are depicted in Table 1. The variables were coded using Equation (1):

$$xi = (X_i - X_0)/\Delta X_i \quad (1)$$

where $xi$ is the independent variable coded value, $X_i$ is the independent variable real value, $X_0$ is the independent variable real value on the center point, and $\Delta X_i$ is the step change value.

**Table 1.** Real and coded values of independent variables according to each $2^3$ full factorial design, for all strains of *Xanthomonas* (*X. axonopodis* pv. *manihotis* IBSBF 290, *X. campestris* pv. *campestris* IBSBF 472, and *Xanthomonas* sp. S6).

| Independent Variable (Concentration on $g \cdot L^{-1}$) | Symbol | Range and Levels | | |
|---|---|---|---|---|
| | | **−1** | **0** | **+1** |
| Sucrose or demerara sugar | $X_1$ | 30 | 50 | 70 |
| $MgSO_4 \cdot 7H_2O$ | $X_2$ | 0.2 | 0.6 | 1.0 |
| $K_2HPO_4$ | $X_3$ | 0.01 | 0.50 | 1.00 |

The individual and interactive effects of carbon source (demerara sugar or sucrose), and $MgSO_4 \cdot 7H_2O$ and $K_2HPO_4$ concentrations on the xanthan production (Y) as response variables were studied. Thereby, a second-order polynomial model using a least-squares method was fitted to evaluate the production (Y), resulting in Equation (2):

$$Y = b_0 + b_{1\times1} + b_{2\times2} + b_{3\times3} + b_{11\times1}{}^2 + b_{22\times2}{}^2 + b_{3\times3}{}^2 + b_{12\times1\times2} + b_{13\times1\times3} + b_{23\times2\times3} \quad (2)$$

where (Y) is the calculated response function; $X_1$, $X_2$, and $X_3$ represent the coded variables (carbon source, $MgSO_4 \cdot 7H_2O$, and $K_2HPO_4$, respectively); and $b_0$, $b_i$, and $b_{ij}$ ($I$, $j$ = 1, 2, 3) are the coefficient estimates.

The software "Statistic" (version 8.0) was applied for regression and graphical analysis of experimental data. The statistical significance of the regression coefficients was determined by the Student *t*-test, while second-order model equations were determined by the *F* test, and the proportions of variance explained by the obtained models were given by the multiple coefficients of determination, $R^2$.

## 3. Results and Discussion

The xanthan production by the three selected strains (*X. axonopodis* pv. *manihotis* IBSBF 290, *X. campestris* pv. *campestris* IBSBF 472, and *Xanthomonas* sp. S6) was developed using six experimental designs (two for each strain). Thus, the $2^3$ full factorial central composite

design with five replicates at the center point comprised a set of 78 experiments. The design matrix of the variables in coded units is depicted in Table 2, as well as the predicted and experimental values of the response factor (xanthan production).

**Table 2.** $2^3$ full factorial central composite design with five replicates at the center point along with the experimental values of xanthan gum production.

| Runs | $X_1$ | $X_2$ | $X_3$ | Production of Xanthan Gum ($g \cdot L^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Strain 290 | | Strain 472 | | Strain S6 | |
| | | | | Sucrose | Demerara Sugar | Sucrose | Demerara Sugar | Sucrose | Demerara Sugar |
| 1 | −1 | −1 | −1 | 0.2993 | 0.3192 | 0.2857 | 0.3931 | 0.1528 | 0.4533 |
| 2 | −1 | −1 | +1 | 0.5166 | 0.2465 | 0.7171 | 1.2739 | 0.0200 | 0.0600 |
| 3 | −1 | +1 | +1 | 0.5992 | 0.2070 | 0.8308 | 1.3075 | 0.9382 | 1.3839 |
| 4 | −1 | +1 | −1 | 0.2847 | 0.2317 | 0.2585 | 0.3619 | 0.5968 | 0.4644 |
| 5 | +1 | +1 | +1 | 0.2784 | 0.3924 | 0.5254 | 0.3750 | 0.0712 | 0.3219 |
| 6 | +1 | −1 | +1 | 0.6840 | 0.2741 | 1.0034 | 0.6109 | 0.0920 | 0.3996 |
| 7 | +1 | −1 | −1 | 0.1127 | 0.2263 | 0.3713 | 0.1373 | 0.0619 | 0.4166 |
| 8 | +1 | +1 | −1 | 0.1454 | 0.1811 | 0.4683 | 0.8674 | 0.9255 | 0.3644 |
| 9 | 0 | 0 | 0 | 0.4926 | 0.3761 | 0.3480 | 0.7854 | 0.4634 | 0.7212 |
| 10 | 0 | 0 | 0 | 0.2859 | 0.4738 | 0.3436 | 0.6200 | 0.5775 | 0.7488 |
| 11 | 0 | 0 | 0 | 0.4182 | 0.4273 | 0.2495 | 0.4562 | 0.6876 | 0.6121 |
| 12 | 0 | 0 | 0 | 0.2750 | 0.3885 | 0.2326 | 0.6213 | 0.4677 | 0.6193 |
| 13 | 0 | 0 | 0 | 0.3010 | 0.4708 | 0.2875 | 0.6315 | 0.7182 | 0.6533 |

The concentration of demerara or sucrose sugar, and the concentration of $MgSO_4 \cdot 7H_2O$ and and $K_2HPO_4$ are important factors that influence the efficiency of the fermentation process for XG production. The conditions of these experiments and the results obtained are listed in Table 2.

As seen in Table 1, the lowest XG production was achieved with the *Xanthomonas* S6 strains in experiment 2 (0.0600 $g \cdot L^{-1}$), where lower concentrations of demerara sugar and $MgSO_4 \cdot 7H_2O$ were used, and higher concentrations of $K_2HPO_4$. Silva et al. [16] also did not find significant effects in relation to the concentration of $MgSO_4 \cdot 7H_2O$; in addition, the presence of $K_2HPO_4$ improved the yield of XG production. The improvement in extraction was observed when the $MgSO_4 \cdot 7H_2O$ concentration increased up to 1 $g \cdot L^{-1}$ in experiment 3, where the highest XG production in this study was obtained for strains 472 and S6 (1.3075 and 1.3839 $g \cdot L^{-1}$, respectively). These results agree with Jesus et al. [19], which achieved higher yields of XG production using the S6 strain supplemented with salts and a hemicellulose fraction obtained by the alkaline hydrolysis of corncob. Furthermore, $MgSO_4 \cdot 7H_2O$ at lower concentrations 0.2 $g \cdot L^{-1}$and 30 $g \cdot L^{-1}$ of demerara sugar showed similar results for strain 472. It was verified that the highest productions of XG for strains *Xanthomonas* 290 and 472, when using sucrose as the main source of carbon, were obtained in the tests containing higher levels of $K_2HPO_4$ and sucrose (experiment 6, 0.6840 and 1.0034 $g \cdot L^{-1}$, respectively). However, for strain 290, the best results were obtained in the conditions applied in the central points (50, 0.6, and 0.50 $g \cdot L^{-1}$ of demerara sugar, $MgSO_4 \cdot 7H_2O$, and $K_2HPO_4$, respectively). In addition, the lowest concentration of XG produced by this strain using demerara sugar was obtained in experiment 8 or more; there were no significant differences between the other experiments. Other authors [44] state in the literature that the most promising culture medium for producing XG is sucrose supplemented with salts. On the other hand, in this study, it is observed that demerara sugar has a higher XG production compared to the experiments with the same conditions using sucrose as a carbon source, making it a very important factor for producing XG.

The quadratic regression models in terms of a coded factor expressed by Equations (3)–(5) represent the XG productions ($Y$) as a function of the sucrose ($X_1$), $MgSO_4 \cdot 7H_2O$

($X_2$), and $K_2HPO_4$ ($X_3$) concentrations reached by each of the *Xanthomonas* strains (290, 472, and S6), respectively.

$$Y = 0.0105\ X_1{}^2 - 0.0599\ X_1 - 0.0381\ X_2 + 0.1545\ X_3 - 0.0551\ X_1X_2 + 0.0215\ X_1X_3 - 0.0426\ X_2X_3 + 0.3545 \quad (3)$$

$$Y = 0.2653\ X_1{}^2 + 0.0345\ X_1 - 0.0368\ X_2 + 0.2116\ X_3 - 0.0584\ X_1X_2 - 0.0393\ X_1X_3 - 0.0543\ X_2X_3 + 0.2922 \quad (4)$$

$$Y = -0.2256\ X_1{}^2 - 0.0696\ X_1 + 0.2756\ X_2 - 0.0769\ X_3 - 0.0649\ X_1X_2 - 0.1291\ X_1X_3 - 0.0513\ X_2X_3 + 0.5823 \quad (5)$$

Regarding the carbon source alteration in fermentation, the quadratic regression models in terms of a coded factor indicating the gum production ($Y$) as a function of demerara sugar ($X_1$), $MgSO_4{\cdot}7H_2O$ ($X_2$), and $K_2HPO_4$ ($X_3$) concentrations are expressed by Equations (6)–(8), also achieved with each studied strain (290, 472, and S6), respectively.

$$Y = -0.1675\ X_1{}^2 + 0.0087\ X_1 - 0.0067\ X_2 + 0.0202\ X_3 + 0.0250\ X_1X_2 + 0.0446\ X_1X_3 + 0.2064\ X_2X_3 + 0.4273 \quad (6)$$

$$Y = 0.0430\ X_1{}^2 - 0.1682\ X_1 + 0.0621\ X_2 + 0.2260\ X_3 + 0.0615\ X_1X_2 - 0.2306\ X_1X_3 - 0.1126\ X_2X_3 + 0.6229 \quad (7)$$

$$Y = -0.1879\ X_1{}^2 - 0.1074\ X_1 + 0.1506\ X_2 + 0.0583\ X_3 - 0.1831\ X_1X_2 - 0.0732\ X_1X_3 + 0.1609\ X_2X_3 + 0.6709 \quad (8)$$

### *3.1. Analysis of Variance (ANOVA)*

The statistical significance of the main interaction effects of the factors on the dependent variable was assessed by the *F* test of the analysis of variance (ANOVA). Tables 3 and 4 depict the experiments with sucrose and demerara sugar, respectively, showing significant results at a 95% confidence level.

**Table 3.** Analysis of variance (ANOVA) for the model regression representing xanthan gum production (Y) using sucrose by *X. axonopodis* pv. *manihotis* 290, *X. campestris* pv. *campestris* 472, and *Xanthomonas* sp. S6, respectively.

| | SS | dF | MS | F | *p*-Value |
|---|---|---|---|---|---|
| Strain 290 | | | | | |
| (1) sucrose (L) | 0.028716 | 1 | 0.028716 | 1.97056 | 0.219346 |
| sucrose (Q) | 0.000339 | 1 | 0.000339 | 0.02327 | 0.884727 |
| (2) $MgSO_4{\cdot}7H_2O$ (L) | 0.011621 | 1 | 0.011621 | 0.79742 | 0.412776 |
| (3) $K_2HPO_4$ (L) | 0.190993 | 1 | 0.190993 | 13.10634 | 0.015215 |
| 1L by 2L | 0.024299 | 1 | 0.024299 | 1.66746 | 0.253072 |
| 1L by 3L | 0.003720 | 1 | 0.003720 | 0.25524 | 0.634884 |
| 2L by 3L | 0.014544 | 1 | 0.014544 | 0.99802 | 0.363654 |
| Error | 0.072863 | 5 | 0.014573 | | |
| Total SS | 0.347094 | 12 | | | |
| Strain 472 | | | | | |
| (1) sucrose (L) | 0.009543 | 1 | 0.009543 | 0.63434 | 0.461891 |
| sucrose (Q) | 0.216603 | 1 | 0.216603 | 14.39851 | 0.012699 |
| (2) $MgSO_4{\cdot}7H_2O$ (L) | 0.010841 | 1 | 0.010841 | 0.72066 | 0.434676 |
| (3) $K_2HPO_4$ (L) | 0.358239 | 1 | 0.358239 | 23.81361 | 0.004553 |
| 1L by 2L | 0.027320 | 1 | 0.027320 | 1.81604 | 0.235625 |
| 1L by 3L | 0.012364 | 1 | 0.012364 | 0.82187 | 0.406201 |
| 2L by 3L | 0.023555 | 1 | 0.023555 | 1.56582 | 0.266171 |
| Error | 0.075217 | 5 | 0.015043 | | |
| Total SS | 0.733682 | 12 | | | |
| Strain S6 | | | | | |
| (1) sucrose (L) | 0.038809 | 1 | 0.038809 | 0.67477 | 0.448775 |
| sucrose (Q) | 0.156573 | 1 | 0.156573 | 2.72232 | 0.159867 |
| (2) $MgSO_4{\cdot}7H_2O$ (L) | 0.607753 | 1 | 0.607753 | 10.56694 | 0.022679 |
| (3) $K_2HPO_4$ (L) | 0.047370 | 1 | 0.047370 | 0.82362 | 0.405737 |
| 1L by 2L | 0.033722 | 1 | 0.033722 | 0.58632 | 0.478413 |
| 1L by 3L | 0.133334 | 1 | 0.133334 | 2.31827 | 0.188357 |
| 2L by 3L | 0.021033 | 1 | 0.021033 | 0.36570 | 0.571750 |
| Error | 0.287573 | 5 | 0.057515 | | |
| Total SS | 1.326168 | 12 | | | |

SS: sum of squares; DF: degrees of freedom; MS: mean square.

**Table 4.** Analysis of variance (ANOVA) for the model regression representing xanthan gum production (Y) using demerara sugar by *X. axonopodis* pv. *manihotis* 290, *X. campestris* pv. *campestris* 472, and *Xanthomonas* sp. S6, respectively.

| | SS | dF | MS | F | *p*-Value |
|---|---|---|---|---|---|
| Strain 290 | | | | | |
| (1) Demerara sugar (L) | 0.000604 | 1 | 0.000604 | 0.30652 | 0.604 |
| Demerara sugar (Q) | 0.086340 | 1 | 0.086340 | 43.83216 | 0.001 |
| (2) $MgSO_4 \cdot 7H_2O$ (L) | 0.000363 | 1 | 0.000363 | 0.18436 | 0.685 |
| (3) $K_2HPO_4$ (L) | 0.003268 | 1 | 0.003268 | 1.65925 | 0.254 |
| 1L by 2L | 0.005005 | 1 | 0.005005 | 2.54089 | 0.172 |
| 1L by 3L | 0.015887 | 1 | 0.015887 | 8.06512 | 0.036 |
| 2L by 3L | 0.005592 | 1 | 0.005592 | 2.83865 | 0.153 |
| Error | 0.009849 | 5 | 0.001970 | | |
| Total SS | 0.126907 | 12 | | | |
| Strain 472 | | | | | |
| (1) Demerara sugar (L) | 0.226397 | 1 | 0.226397 | 6.05021 | 0.057 |
| Demerara sugar (Q) | 0.005688 | 1 | 0.005688 | 0.15200 | 0.713 |
| (2) $MgSO_4 \cdot 7H_2O$ (L) | 0.030826 | 1 | 0.030826 | 0.82380 | 0.406 |
| (3) $K_2HPO_4$ (L) | 0.408427 | 1 | 0.408427 | 10.91476 | 0.021 |
| 1L by 2L | 0.030233 | 1 | 0.030233 | 0.80795 | 0.410 |
| 1L by 3L | 0.425595 | 1 | 0.425595 | 11.37356 | 0.020 |
| 2L by 3L | 0.101520 | 1 | 0.101520 | 2.71301 | 0.160 |
| Error | 0.187099 | 5 | 0.037420 | | |
| Total SS | 1.415786 | 12 | | | |
| Strain S6 | | | | | |
| (1) Demerara sugar (L) | 0.092257 | 1 | 0.092257 | 1.930789 | 0.223 |
| Demerara sugar (Q) | 0.108667 | 1 | 0.108667 | 2.274231 | 0.192 |
| (2) $MgSO_4 \cdot 7H_2O$ (L) | 0.181533 | 1 | 0.181533 | 3.799212 | 0.109 |
| (3) $K_2HPO_4$ (L) | 0.027226 | 1 | 0.027226 | 0.569801 | 0.484 |
| 1L by 2L | 0.268242 | 1 | 0.268242 | 5.613882 | 0.064 |
| 1L by 3L | 0.042881 | 1 | 0.042881 | 0.897424 | 0.387 |
| 2L by 3L | 0.207143 | 1 | 0.207143 | 4.335177 | 0.092 |
| Error | 0.238909 | 5 | 0.047782 | | |
| Total SS | 1.166857 | 12 | | | |

SS: sum of squares; DF: degrees of freedom; MS: mean square.

The *Xanthomonas* growth time can vary according to the fermentation conditions. Xanthan production is closely related to the carbon source consumption up to its maximum concentration. Afterward, the enzymatic polysaccharide hydrolysis starts to restore the carbon source in the culture medium as an attempt to maintain bacterial viability [45]. Therefore, the carbon source is a critical factor in any bioproduct formation, including XG [46]. Thereby, it is noteworthy to evaluate each carbon source interaction with respect to the other variables in the medium composition independently, to establish the carbon-source–micronutrient interactions, such as $MgSO_4 \cdot 7H_2O$ and $K_2HPO_4$ [14].

The statistical study using an ANOVA-linked *Fisher* (*F*) test was applied to determine the significant variables, whose significance degree was ranked based on the F-ratio value. In fact, the greater the magnitude of the *F* value and the lower the "Prob < *F*" value, the more significantly displayed the corresponding model and individual coefficient. The *p*-value serves as a tool to check the significance of each of the coefficients, also indicating the interaction strength between the parameters. Low *p*-values (<0.05) denote a greater correlation of the significance between corresponding coefficients [32,47].

Hence, in the study using sucrose as a carbon source (Table 3), a significant linear effect ($p < 0.05$) of $K_2HPO_4$ was observed with strain 290 ($p = 0.015$) and strain 472 ($p = 0.004$), and of $MgSO_4 \cdot 7H_2O$ with strain S6 ($p = 0.023$). Silva et al. [16] and Niknezhad et al. [47] also reported potassium as a significant factor in fermentations using cheese whey, $K_2HPO_4$, and $MgSO_4$ for xanthan production. Notwithstanding, Umashankar et al. [48] observed the nutrient influence on *X. campestris* growth under a high phosphate concentration, which

inhibited gum production. Nonetheless, the sucrose quadratic term coefficients ($p = 0.013$) were significant for strain 472, indicating that both the concentration and carbon source type are important for the cells during the fermentation process for xanthan production. This result is consistent with that reported by Demirci et al. [21], verifying the significant glucose effect on polymer production.

The demerara sugar concentration evaluation depicted in Table 4 shows the ANOVA statistical analysis concerning the influence of this sugar concentration, $K_2HPO_4$, and $MgSO_4 \cdot 7H_2O$ on xanthan production. The effects of $MgSO_4 \cdot 7H_2O$ associated with the demerara sugar rate displayed no statistical significance for the strains tested. However, the significant effect ($p < 0.05$) on the response for strain 290 was the demerara sugar concentration quadratic term ($p = 0.001$), while the linear term of the $K_2HPO_4$ concentration ($p = 0.021$) was significant for strain 472. Furthermore, the interaction between demerara sugar and the $K_2HPO_4$ concentration exhibited a significant effect ($p = 0.036$ and $p = 0.020$) for the 290 and 472 strains, respectively.

The measures of the models' goodness-of-fit were confirmed by the determination coefficients ($R^2$), with $R^2$ values of 0.79008, 0.89748, and 0.78316 by the 290, 472, and S6 strains, respectively, for the sucrose experiments, while for the demerara sugar experiments, the $R^2$ values for the 290, 472, and S6 strains were 0.92239, 0.86785, and 0.79525, respectively. The $R^2$ value provides a variability measure in the observed response values, which can be explained by the experimental parameters and their interactions. $R^2$ values close to 1 indicate the best correlation between the experimental and predicted values and the better predictive response model [32]. Therefore, the $R^2$ predicted values > 0.78 for all strains pointed to a reasonable agreement between the experimental and predicted values for xanthan production.

### *3.2. Response Surface Methodology (RSM)*

The response surface methodology (RSM) is often applied in order to provide effective tools for optimization since it involves many critical factors for determining high accuracy [32,49]. Figures 2 and 3 represent the response surfaces of the regression study using sucrose.

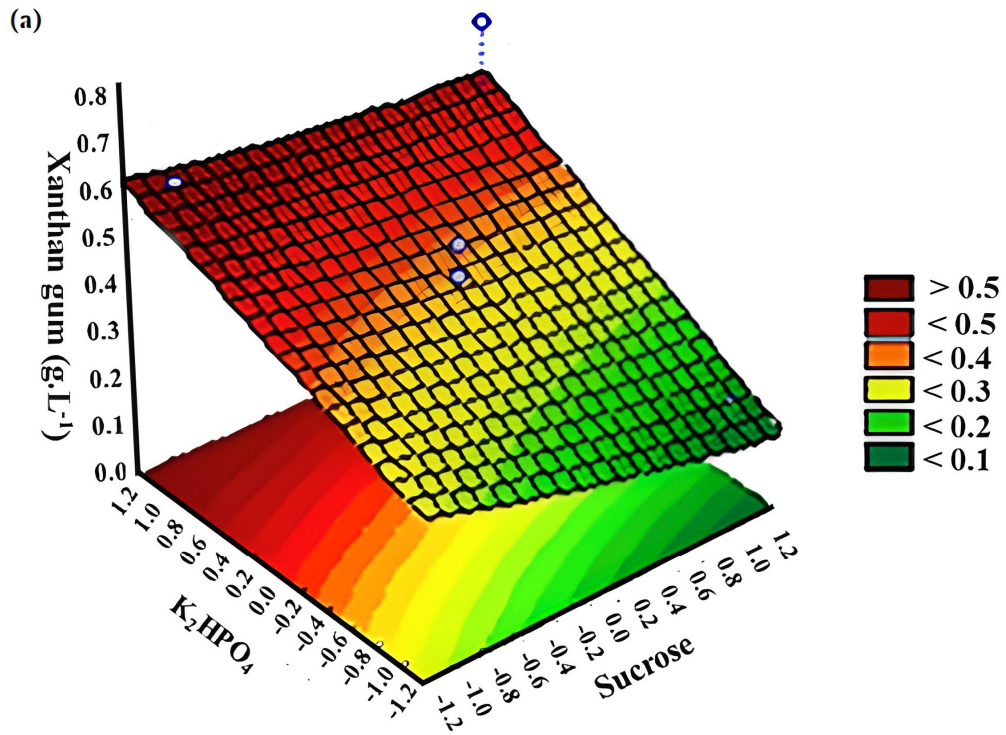


**Figure 2.** *Cont.*

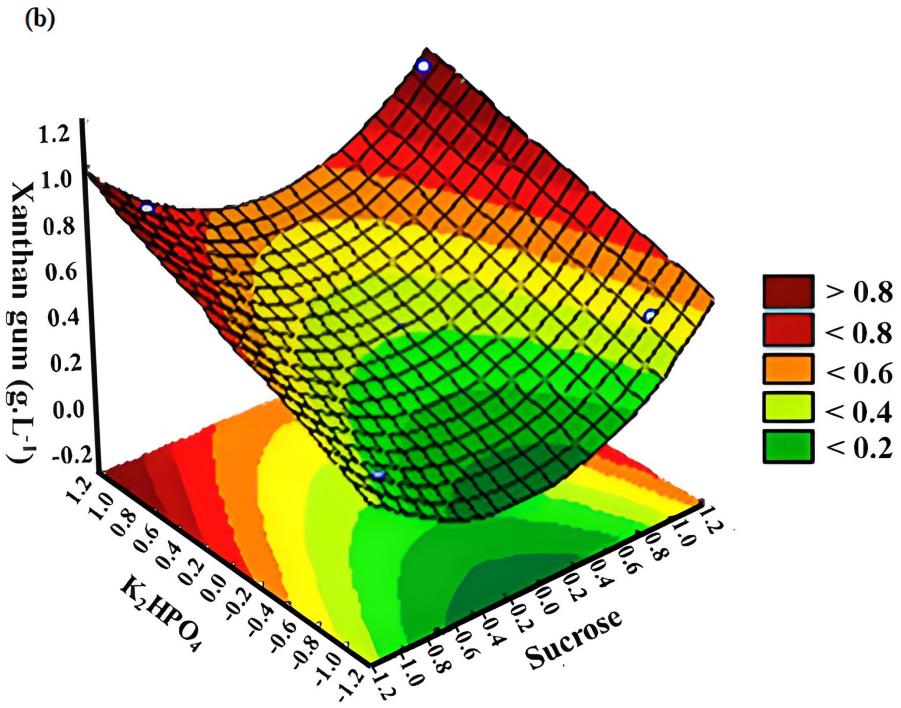


**Figure 2.** Response surface plots depicting the sucrose and $K_2HPO_4$ concentration effect and their mutual effects on xanthan production by (**a**) *X. axonopodis* pv. *manihotis* 290 and (**b**) *X. campestris* pv. *campestris* 472.

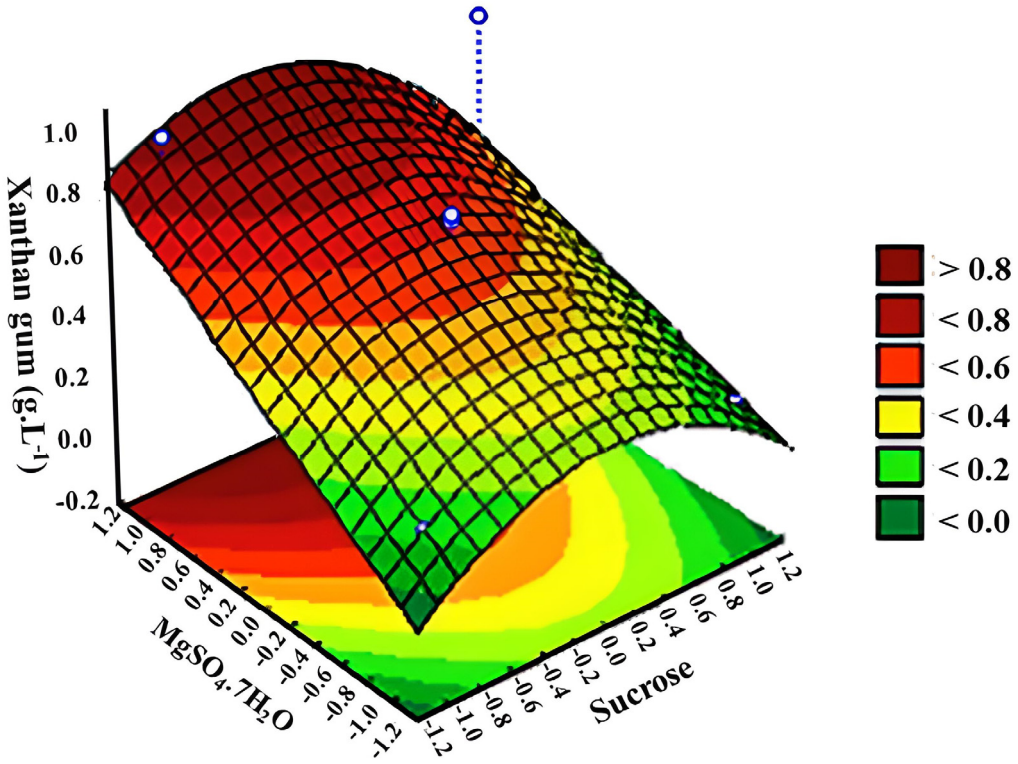


**Figure 3.** Response surface plot representing the sucrose and $MgSO_4 \cdot 7H_2O$ concentration effect and their mutual effects on xanthan production by *Xanthomonas* sp. S6.

Figure 2a,b show the response surface plots for xanthan production at different concentrations of sucrose and $K_2HPO_4$ for the *Xanthomonas* 290 and 472 strains, respectively. Likewise, Figure 2 depicts the relationship between the $MgSO_4 \cdot 7H_2O$ concentrations with

sucrose for the S6 strain. According to the response surface plots, the xanthan production yield rises with the increased $K_2HPO_4$ concentration relative to the 290 and 472 strains, while an increased $MgSO_4 \cdot 7H_2O$ concentration promoted a higher yield by the S6 strain. In both cases, the independent variables $MgSO_4 \cdot 7H_2O$ and $K_2HPO_4$ significantly affected xanthan production in each *Xanthomonas* strain.

The results show the importance of screening the *Xanthomonas* strains in association with a fermentation variable study since the culture medium composition can promote different behaviors on the strains used for production. Therefore, the response surface methodology aimed to detect the experimental parameters responsible for signals superior to those caused by noise. The phosphate addition to the medium can increase xanthan gum production by acting as a buffering agent, minimizing pH fluctuations [50].

Concerning Figure 4a,b, the response surface displays the relationship between the $K_2HPO_4$ concentrations and demerara sugar for the *Xanthomonas* 290 and 472 strains, respectively. High $K_2HPO_4$ concentrations provided a significant xanthan production increase, previously observed in fermentations using sucrose. Moreover, it was observed that the *Xanthomonas* strain 290 grown in demerara sugar, even with a low $K_2HPO_4$ content, kept gum production close to its highest level when grown in demerara sugar at the central point level. The data suggest that the demerara sugar and $K_2HPO_4$ use provided a high potassium content, affecting gum production. The experiment performed with maximum values of demerara sugar and $MgSO_4 \cdot 7H_2O$ and a minimum value of $K_2HPO_4$ displayed the third-largest gum production by the *Xanthomonas* 472 strain. Therefore, demerara sugar could supply the potassium amount required for gum production.

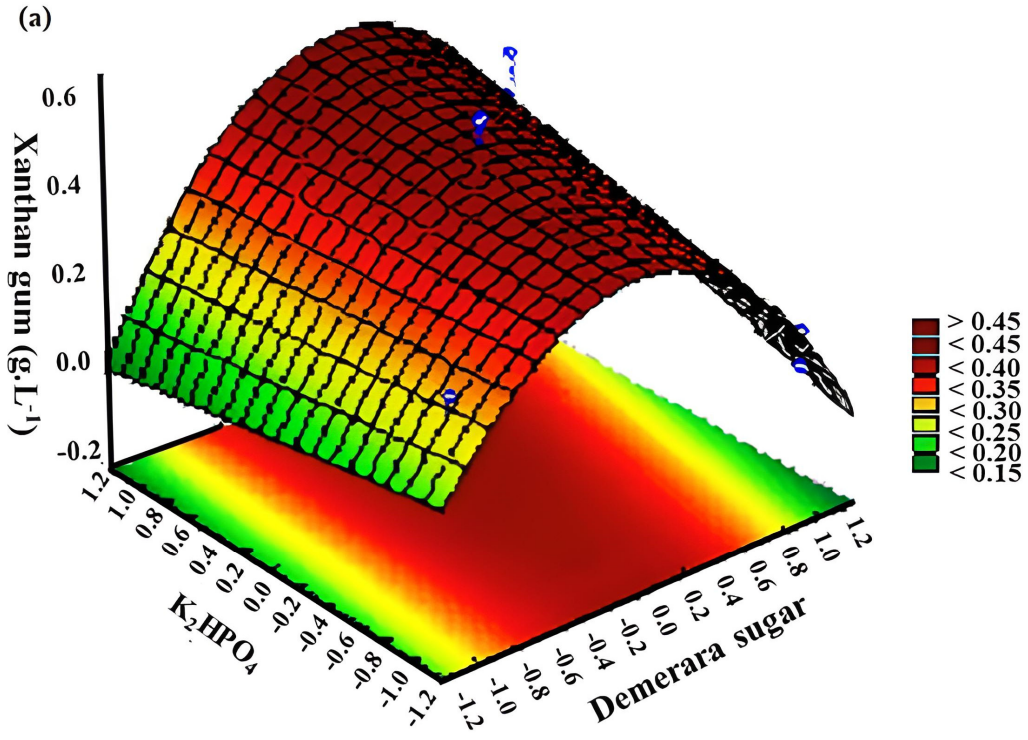


**Figure 4.** *Cont.*

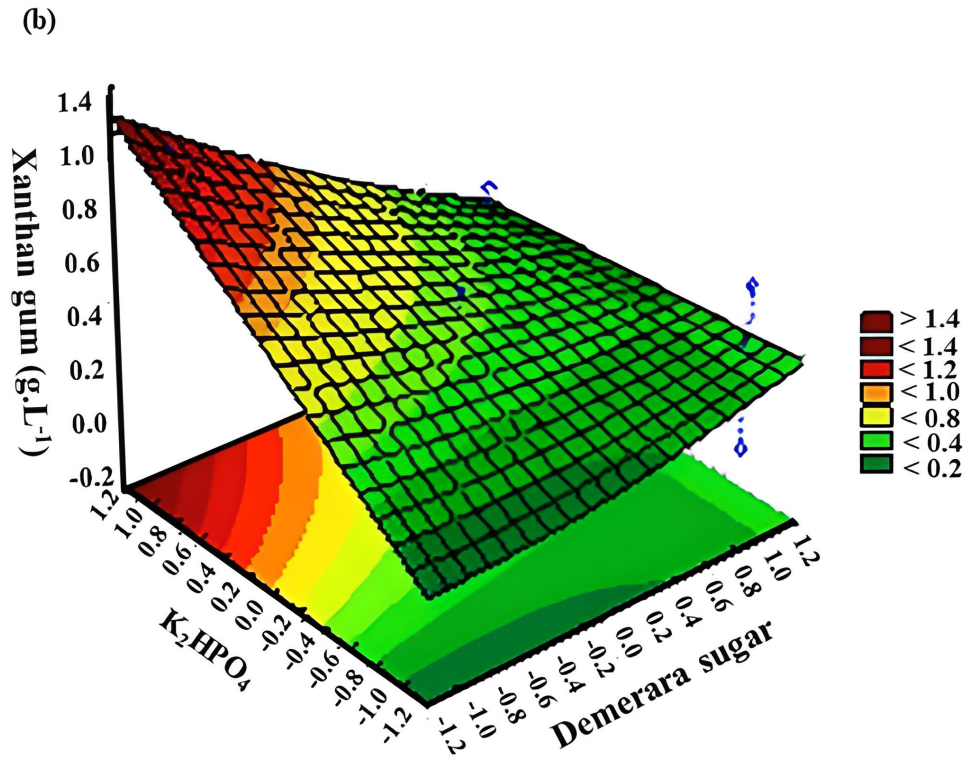


**Figure 4.** Response surface plots depicting the demerara sugar and $K_2HPO$ concentration effect and their mutual effects on xanthan production by (**a**) *X. axonopodis* pv. *manihotis* 290 and (**b**) *X. campestris* pv. *campestris* 472.

Overall, these results are supported by Faria et al. [51] on the influence of the initial sucrose concentration on xanthan production, and by García-Ochoa et al. [4] on the effects of nitrogen, phosphorus, and magnesium on bacterial growth and polymer production. Micronutrients may be required for pyruvilation, although the literature reports several results regarding its effect on the pyruvilation degree in the xanthan molecular structure [14]. Pyruvate influences the polymer–polymer interaction increase related to the affinity with the neighboring xanthan molecules, promoting more viscous aqueous solutions [52].

The significant effects of $K_2HPO_4$ were similar to those described by Silva et al. [16] and Mesomo et al. [53] These authors evaluated the combined effects of $K_2HPO_4$ and $MgSO_4 \cdot 7H_2O$ with the cheese whey addition, observing the higher xanthan production with the maximum $K_2HPO_4$ supplementation by *X. campestris* pv. *mangiferaeindicae* 1230 and *X. campestris* pv. *manihotis* 1182. Kalogiannis et al. [50] also reported the maximal production using pretreated beet molasses (carbon source), supplemented with $K_2HPO_4$, yeast extract, and Triton 80. Moreover, potassium can significantly increase the xanthan viscosity [54] which, in the form of $K_2HPO_4$ and $KH_2PO_4$, are significant medium components, acting as buffering agents as well as nutrients for *Xanthomonas* growth [55].

Despite evidence showing the influence of the microbial strain, time, and fermentation medium composition on XG production, no alternative medium has even replaced the use of sucrose or glucose with a significant effect on the polysaccharide quality and productivity. Nevertheless, the results with demerara sugar showed its efficiency as a carbon source in gum production due to its nutritional richness, which can partially replace the evaluated salt addition. Even so, further studies with demerara sugar or other potential substrates are required, whose nutrient content can provide operational and economic advantages for a xanthan production process since the analyzed variables are significant for the polymer-producing strains.

## 4. Conclusions

This study evaluated the possibility of supplementing the fermentation medium with mineral salts, using demerara sugar or sucrose as substrates, in XG production. Thereby, the sucrose or demerara sugar, $K_2HPO_4$, and $MgSO_4 \cdot 7H_2O$ concentration effects on its production were explored by an experimental design and a response surface analysis. The proposed model studies pointed out a reasonable experimental agreement for xanthan production, indicating specific nutritional requirements for each *Xanthomonas* strain. The three selected strains displayed a different influence from the fermented conditions for xanthan production. Statistical significance values suggested that the sugar concentration and $K_2HPO_4$ played an important role for this production by the *Xanthomonas* 472 strain, using sucrose or demerara sugar as a carbon source. Regarding the S6 strain, $MgSO_4 \cdot 7H_2O$ played a significant role in gum synthesis with sucrose but not with demerara as a carbon source. With strain 290, the demerara sugar concentration increases the XG but with sucrose as the carbon source, the production is higher with $K_2HPO_4$. The evaluated salts are important factors for XG production, and the demerara sugar can partially replace this mineral salt requirement. Economically, the experimental data show the relevance of demerara sugar as an efficient and low-cost alternative source of carbon in the production of XG compared to a carbon source used industrially. These results contribute to the production of XG through sustainable biotechnology, efficient in the context of the circular bioeconomy.

**Author Contributions:** Conceptualization, D.P.S., D.S.R. and L.C.R.; methodology, L.C.R., A.S.F.-J., E.S.N. and K.S.S.; formal analysis, D.P.S., D.S.R. and L.C.R.; investigation, L.C.R., A.S.F.-J., E.S.N., and K.S.S.; resources, D.P.S., P.P., and D.S.R.; data curation, D.P.S., D.S.R., L.C.R., J.A.T., P.P. and M.S.J.; writing—original draft preparation, L.C.R. and F.F.P.; writing—review and editing, D.P.S., D.S.R., P.P. and M.S.J.; supervision, D.P.S. and D.S.R.; project administration, D.P.S. and D.S.R. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** All data generated or analyzed during this study are included in this published article.

**Acknowledgments:** The authors acknowledge the financial assistance from the Brazilian research funding agencies including the Co-ordination for the Improvement of Higher Education Personnel (CAPES) under Finance Code 001, a Brazilian foundation within the Ministry of Education (MEC); the National Council for Scientific and Technological Development (CNPq), a Brazilian foundation associated to the Ministry of Science, Technology, and Innovations (MCTI); the Foundation of Support to Research and Technological Innovation of the State of Sergipe (FAPITEC/SE); and the Regional Co-operative of Agrarian Reform Settlers of Sergipe's Semi-Arid. The authors acknowledge the Foundation for Science and Technology (FCT, Portugal) for the financial support to the CISAS UIDB/05937/2020 and UIDP/05937/2020, including the postdoc grant and the contract of the two authors.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Sharma, A.; Gautam, S.; Wadhawan, S. *Xanthomonas. Encyclopedia of Food Microbiology*, 2nd ed.; Elsevier: Amsterdam, The Netherlands, 2014; pp. 811–817. [CrossRef]
2. Dzionek, A.; Wojcieszyńska, D.; Guzik, U. Use of xanthan gum for whole cell immobilization and its impact in bioremediation—A review. *Bioresour. Technol.* **2022**, *351*, 126918. [CrossRef] [PubMed]
3. Heyman, B.; De Vos, W.H.; Depypere, F.; Van der Meeren, P.; Dewettinck, K. Guar and xanthan gum differentially affect shear induced breakdown of native waxy maize starch. *Food Hydrocoll.* **2014**, *35*, 546–556. [CrossRef]
4. García-Ochoa, F.; Santos, V.E.; Casas, J.A.; Gomez, E. Xanthan gum: Production, recovery, and properties. *Biotechnol. Adv.* **2000**, *18*, 549–579. [CrossRef] [PubMed]

5. Abu Elella, M.H.; Goda, E.S.; Gab-Allah, M.A.; Hong, S.E.; Pandit, B.; Lee, S.; Gamal, H.; Rehman, A.U.; Yoon, K.R. Xanthan gum-derived materials for applications in environment and eco-friendly materials: A review. *J. Environ. Chem. Eng.* **2020**, *9*, 104702. [CrossRef]
6. Furtado, I.F.S.P.C.; Sydney, E.B.; Rodrigues, S.A.; Sydney, A.C.N. Xanthan gum: Applications, challenges, and advantages of this asset of biotechnological origin. *Biotechnol. Res. Innov.* **2022**, *6*, e202204. [CrossRef]
7. Bhat, I.M.; Wani, S.M.; Mir, S.A.; Masoodi, F. Advances in xanthan gum production, modifications and its applications. *Biocatal. Agric. Biotechnol.* **2022**, *42*, 102328. [CrossRef]
8. Al-Muhanna, M.K.; Anwar, N.; Hasnain, S.; Nayak, A.K. Chapter 1–Synthesis of tailor-made polysaccharides: An overview. In *Tailor-Made Polysaccharides in Drug Delivery*; Elsevier: Amsterdam, The Netherlands, 2023; pp. 1–27. [CrossRef]
9. Patel, J.; Maji, B.; Moorthy, N.S.H.N.; Maiti, S. Xanthan gum derivatives: Review of synthesis, properties and diverse applications. *RSC Adv.* **2020**, *10*, 27103–27136. [CrossRef]
10. Şen, E.; Demirci, A.S.; Palabiyik, I. Xanthan gum characterization and production kinetics from pomace of *Vitis vinifera*. *J. Food Process. Preserv.* **2022**, *46*, e17098. [CrossRef]
11. Xanthan Gum Market Will Be Worth USD 730.39 Million by 2030. Available online: https://www.marketresearchfuture.com/reports/xanthan-gum-market-748 (accessed on 13 February 2023).
12. Maurya, D.K.; Kumar, A.; Chaurasiya, U.; Hussain, T.; Singh, S.K. Modern era of microbial biotechnology: Opportunities and future prospects. *Microbiomes Plant Health Panoply Appl.* **2020**, *1*, 317–343. [CrossRef]
13. Pirsa, S.; Hafezi, K. Hydrocolloids: Structure, preparation method, and application in food industry. *Food Chem.* **2023**, *399*, 133967. [CrossRef]
14. Chaitali, M.; Kapadi, M.; Suraishkumar, G.K.; Gudi, R.D. Productivity Improvement in Xanthan Gum Fermentation Using Multiple Substrate Optimization. *Biotechnol. Prog.* **2008**, *19*, 1190–1198. [CrossRef]
15. Habibi, H.; Khosravi-Darani, K. Effective variables on production and structure of xanthan gum and its food applications: A review. *Biocatal. Agric. Biotechnol.* **2017**, *10*, 130–140. [CrossRef]
16. Silva, M.F.; Fornari, R.C.; Mazutti, M.A.; de Oliveira, D.; Padilha, F.F.; Cichoski, A.J.; Cansian, R.L.; Di Luccio, M.; Treichel, H. Production and characterization of xantham gum by *Xanthomonas campestris* using cheese whey as sole carbon source. *J. Food Eng.* **2009**, *90*, 119–123. [CrossRef]
17. Berninger, T.; Dietz, N.; González López, Ó. Water-soluble polymers in agriculture: Xanthan gum as eco-friendly alternative to synthetics. *Microb. Biotechnol.* **2021**, *14*, 1881–1896. [CrossRef]
18. Rottava, I.; Batesini, G.; Silva, M.F.; Lerin, L.A.; de Oliveira, D.; Padilha, F.F.; Toniazzo, G.; Mossi, A.; Cansian, R.L.; Di Luccio, M.; et al. Xanthan gum production and rheological behavior using different strains of *Xanthomonas* sp. *Carbohydr. Polym.* **2009**, *77*, 65–71. [CrossRef]
19. Jesus, M.; Mata, F.; Batista, R.A.; Ruzene, D.S.; Albuquerque-Júnior, R.; Cardoso, J.C.; Vaz-Velho, M.; Pires, P.; Padilha, F.F.; Silva, D.P. Corncob as Carbon Source in the Production of Xanthan Gum in Different Strains *Xanthomonas* sp. *Sustainability* **2023**, *15*, 2287. [CrossRef]
20. da Silva, J.A.; Cardoso, L.G.; de Jesus Assis, D.; Gomes, G.V.P.; Oliveira, M.B.P.P.; de Souza, C.O.; Druzian, J.I. Xanthan Gum Production by *Xanthomonas campestris* pv campestris IBSBF 1866 and 1867 from Lignocellulosic Agroindustrial Wastes. *Appl. Biochem. Biotechnol.* **2018**, *186*, 750–763. [CrossRef]
21. Demirci, A.S.; Palabiyik, I.; Apaydın, D.; Mirik, M.; Gumus, T. Xanthan gum biosynthesis using *Xanthomonas* isolates from waste bread: Process optimization and fermentation kinetics. *LWT* **2018**, *101*, 40–47. [CrossRef]
22. Cuadrado-Osorio, P.D.; Ramírez-Mejía, J.M.; Mejía-Avellaneda, L.F.; Mesa, L.; Bautista, E.J. Agro-industrial residues for microbial bioproducts: A key booster for bioeconomy. *Bioresour. Technol. Rep.* **2022**, *20*, 101232. [CrossRef]
23. Bernardo, R.; Lourenzani, W.L.; Satolo, E.G.; Caldas, M.M. Analysis of the agricultural productivity of the sugarcane crop in regions of new agricultural expansions of sugarcane. *Gestão Produção* **2019**, *26*. [CrossRef]
24. Faria, S.; Petkowicz, C.L.D.O.; de Morais, S.A.L.; Terrones, M.G.H.; de Resende, M.M.; de França, F.P.; Cardoso, V.L. Characterization of xanthan gum produced from sugar cane broth. *Carbohydr. Polym.* **2011**, *86*, 469–476. [CrossRef]
25. Zhu, Z.; Xie, C.; Li, W.; Hang, F.; Li, K.; Shi, C.; Doherty, W.O. Nutritional and antioxidant properties of non-centrifugal cane sugar derived from membrane clarified juice. *LWT* **2020**, *131*, 109717. [CrossRef]
26. Baikow, V. *Manufacture and Refining of Raw Cane Sugar*; Elsevier: New York, NY, USA, 2013; pp. 1–588.
27. Jaffé, W.R. Nutritional and functional components of non centrifugal cane sugar: A compilation of the data from the analytical literature. *J. Food Compos. Anal.* **2015**, *43*, 194–202. [CrossRef]
28. D'Adamo, I.; Gastaldi, M.; Morone, P.; Rosa, P.; Sassanelli, C.; Settembre-Blundo, D.; Shen, Y. Bioeconomy of Sustainability: Drivers, Opportunities and Policy Implications. *Sustainability* **2021**, *14*, 200. [CrossRef]
29. Letisse, F.; Lindley, N.; Roux, G. Development of a Phenomenological Modeling Approach for Prediction of Growth and Xanthan Gum Production Using *Xanthomonas campestris*. *Biotechnol. Prog.* **2003**, *19*, 822–827. [CrossRef]
30. Soleymanpour, Z.; Nikzad, M.; Talebnia, F.; Niknezhad, V. Xanthan gum production from acid hydrolyzed broomcorn stem as a sole carbon source by *Xanthomonas campestris*. *3 Biotech* **2018**, *8*, 296. [CrossRef]
31. Box, G.E.P.; Hunter, J.S.; Hunter, W.G. *LIBRO: Statistics Experimenters*; Wiley: New York, NY, USA, 2009; pp. 1–655.

32. Vaishnav, A.; Upadhyay, K.; Koradiya, M.; Tipre, D.; Dave, S. Valorisation of fruit waste for enhanced exopolysaccharide production by *Xanthomonas campestries* using statistical optimisation of medium and process. *Food Biosci.* **2022**, *46*, 101608. [CrossRef]
33. Mohsin, A.; Akyliyaevna, K.A.; Zaman, W.Q.; Hussain, M.H.; Mohsin, M.Z.; Al-Rashed, S.; Tan, X.; Tian, X.; Aida, K.; Tariq, M.; et al. Kinetically modelled approach of xanthan production using different carbon sources: A study on molecular weight and rheological properties of xanthan. *Int. J. Biol. Macromol.* **2021**, *193*, 1226–1236. [CrossRef]
34. Gunasekar, V.; Reshma, K.; Treesa, G.; Gowdhaman, D.; Ponnusami, V. Xanthan from sulphuric acid treated tapioca pulp: Influence of acid concentration on xanthan fermentation. *Carbohydr. Polym.* **2014**, *102*, 669–673. [CrossRef]
35. Prajapati, J.; Panchal, R.; Patel, D.; Goswami, D. Production and characterization of xanthan gum by *Xanthomonas campestris* using sugarcane bagasse as sole carbon source. In *Biotechnology and Biological Sciences: Proceedings of the 3rd International Conference of Biotechnology and Biological Sciences (BIOSPECTRUM 2019), Kolkata, India, 8–10 August 2019*; Taylor and Francis: London, UK, 2019; pp. 363–367. [CrossRef]
36. Mohsin, A.; Zhang, K.; Hu, J.; Rehman, S.U.; Tariq, M.; Zaman, W.Q.; Khan, I.M.; Zhuang, Y.; Guo, M. Optimized biosynthesis of xanthan via effective valorization of orange peels using response surface methodology: A kinetic model approach. *Carbohydr. Polym.* **2018**, *181*, 793–800. [CrossRef]
37. Li, P.; Zeng, Y.; Xie, Y.; Li, X.; Kang, Y.; Wang, Y.; Xie, T.; Zhang, Y. Effect of pretreatment on the enzymatic hydrolysis of kitchen waste for xanthan production. *Bioresour. Technol.* **2017**, *223*, 84–90. [CrossRef]
38. Demirci, A.S. Xanthan Gum Production from Hydrolyzed Rice Bran as a Carbon Source by *Xanthomonas* spp. *Microbiol. Biotechnol. Lett.* **2012**, *40*, 356–363. [CrossRef]
39. Ozdal, M.; Kurbanoglu, E.B. Valorisation of chicken feathers for xanthan gum production using *Xanthomonas campestris* MO-03. *J. Genet. Eng. Biotechnol.* **2018**, *16*, 259–263. [CrossRef]
40. Soltaninejad, A.; Jazini, M.; Karimi, K. Biorefinery for efficient xanthan gum, ethanol, and biogas production from potato crop residues. *Biomass Bioenergy* **2022**, *158*, 106354. [CrossRef]
41. Rončević, Z.; Grahovac, J.; Dodić, S.; Vučurović, D.; Dodić, J. Utilisation of winery wastewater for xanthan production in stirred tank bioreactor: Bioprocess modelling and optimisation. *Food Bioprod. Process.* **2019**, *117*, 113–125. [CrossRef]
42. Brandão, L.V.; Assis, D.; Lopez, J.A.; Espiridião, M.C.A.; Echevarria, E.M.; Druzian, J.I. Bioconversion from crude glycerin by *Xanthomonas campestris* 2103: Xanthan production and characterization. *Braz. J. Chem. Eng.* **2013**, *30*, 737–746. [CrossRef]
43. Mishra, A.; Mohite, A.M.; Sharma, N. Influence of particle size on physical, mechanical, thermal, and morphological properties of tamarind- fenugreek mucilage biodegradable films. *Polym. Bull.* **2022**, *80*, 3119–3133. [CrossRef]
44. Haas, I.C.D.S.; Toaldo, I.M.; Burin, V.M.; Bordignon-Luiz, M.T. Extraction optimization for polyphenolic profiling and bioactive enrichment of extractives of non-pomace residue from grape processing. *Ind. Crop. Prod.* **2018**, *112*, 593–601. [CrossRef]
45. Miranda, A.L.; Costa, S.S.; Assis, D.D.J.; Jesus, C.S.; Gil Guimarães, A.; Druzian, J.I. Influence of strain and fermentation time on the production, composition, and properties of xanthan gum. *J. Appl. Polym. Sci.* **2019**, *137*, 48557. [CrossRef]
46. Trindade, R.A.; Munhoz, A.P.; Burkert, C.A. Impact of a carbon source and stress conditions on some properties of xanthan gum produced by *Xanthomonas campestris* pv. *mangiferaeindicae*. *Biocatal. Agric. Biotechnol.* **2018**, *15*, 167–172. [CrossRef]
47. Niknezhad, S.V.; Asadollahi, M.A.; Zamani, A.; Biria, D. Production of xanthan gum by free and immobilized cells of *Xanthomonas campestris* and *Xanthomonas pelargonii*. *Int. J. Biol. Macromol.* **2016**, *82*, 751–756. [CrossRef] [PubMed]
48. Umashankar, H.; Annadurai, G.; Chellapandian, M.; Krishnan, M.R.V. Influence of Nutrients on Cell Growth and Xanthan Production by *Xanthomonas campestris*. *Bioprocess Eng.* **1996**, *14*, 307–309. [CrossRef]
49. Rabiya, R.; Sen, R. Artificial intelligence driven advanced optimization strategy vis-à-vis response surface optimization of production medium: Bacterial exopolysaccharide production as a case-study. *Biochem. Eng. J.* **2021**, *178*, 108271. [CrossRef]
50. Kalogiannis, S.; Iakovidou, G.; Liakopoulou-Kyriakides, M.; Kyriakidis, D.A.; Skaracis, G.N. Optimization of xanthan gum production by *Xanthomonas campestris* grown in molasses. *Process. Biochem.* **2003**, *39*, 249–256. [CrossRef]
51. Faria, S.; Vieira, P.A.; Resende, M.M.; Ribeiro, E.J.; Cardoso, V.L. Application of a model using the phenomenological approach for prediction of growth and xanthan gum production with sugar cane broth in a batch process. *LWT* **2010**, *43*, 498–506. [CrossRef]
52. Li, R.; Feke, D.L. Rheological and kinetic study of the ultrasonic degradation of xanthan gum in aqueous solution: Effects of pyruvate group. *Carbohydr. Polym.* **2015**, *124*, 216–221. [CrossRef]
53. Mesomo, M.; Silva, M.F.; Boni, G.; Padilha, F.F.; Mazutti, M.; Mossi, A.; de Oliveira, D.; Cansian, R.L.; Di Luccio, M.; Treichel, H. Xanthan gum produced by *Xanthomonas campestris* from cheese whey: Production optimisation and rheological characterisation. *J. Sci. Food Agric.* **2009**, *89*, 2440–2445. [CrossRef]
54. Xu, L.; Dong, M.; Gong, H.; Sun, M.; Li, Y. Effects of inorganic cations on the rheology of aqueous welan, xanthan, gellan solutions and their mixtures. *Carbohydr. Polym.* **2015**, *121*, 147–154. [CrossRef]
55. Katherine, R.F.; Muthukumaran, C.; Sharmila, G.; Kumar, N.M.; Tamilarasan, K.; Jaiganesh, R. Xanthan gum production using jackfruit-seed-powder-based medium: Optimization and characterization. *3 Biotech* **2017**, *7*, 248. [CrossRef]


**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.